International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 7, Issue 2, Apr 2017, 15-22

TRANS STELLAR
•Journal Publications •Research Consultancy

POLLEN STUDIES IN KIWIFRUIT (ACTINIDIA DELICIOSA CHIVE.)

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ABSTRACT

In the present study difference in the shape and size of the pollen grains was observed under different condition. Maximum length of pollen (186.5µ and 119.47µ in both male and hermaphrodite plants respectively) was recorded under dry condition and the pollen were look like bar shaped structure. When the pollen grains are hydrated, they took the spherical shape. Male flower pollen grains contain trinucleate pollens where as hermaphrodite pollen grains contain shriveled inner cell content. The pollen of both male and hermaphrodite flower shows 100 % viability on the day of anthesis under natural condition. The pollen grains remain viable for 3 days but in pistillate flowers there is a rapid decrease in the viability compared to male pollen grains under natural condition. The maximum pollen germination (91.60%in male flower and 25.20% in hermaphrodite flower) and longest pollen tube (2483.10µ and 550.02µ in male and hermaphrodite flower respectively) was recorded with the combination of 0.5% boric acid and 15% sucrose solution.

KEYWORDS: Pollen, Sucrose, Viability, Longevity, Anthesis

Received: Jan 03, 2017; **Accepted:** Feb 08, 2017; **Published:** Feb 11, 2017; **Paper Id.:** IJASRAPR20173

Original Article

INTRODUCTION

Kiwifruit (*Actinidia deliciosa*) belongs to the family actinidiaceae. It is also known as chinese goosberry and national fruit of New Zeland. In India, kiwifruit was introduced in 1960s to Lal Bhag garden, Bengaluru as an ornamental plant. Kiwi is mostly grown in the mid hills of Himachal Pradesh, Uttarakhand, J & K, Sikkim, Meghalaya, Arunachal Pradesh and Kerala. Having been very newly introduced in the country estimates of area and production have not yet become available. In Uttarakhand, the area under its cultivation is 117 ha with annual production of 555 metric tons (NHB, 2013).

The plant is highly androdioecious in nature in which both male and hermaphrodite flowers were born on different plants. Kiwi plants are highly woody perennial veins which are known as liana. The plants have alternate phyllotaxy with compound leaves. The plants are highly deciduous in nature which means they usually shed their leaves during the winter season. The kiwiplants requires minimum of 700 - 800 hours of chilling requirement in which the plants faces a temperature of below 7^{o} C. Both female and hermaphrodite flower required same type of conditions for the flowering.

Fresh Kiwi fruit have high nutritional value (per 100 g) i.e., Energy 255kJ (61 kcal), Carbohydrates 14.66g, Sugars 8.99g, Dietary fiber 3.0g, Fat 0.52g, Protein 1.14g, lutein and zeaxanthin 122µg, Thiamine (Vitamin B1) 0.027mg, Riboflavin (Vitamin B2) 0.025mg, Niacin (Vitamin B3) 0.341mg, Vitamin B6 0.63mg, Folate (Vitamin B9) 25µg, Vitamin C 92.7mg, Vitamin E 1.5mg, Vitamin K 40.3µg, Calcium 34mg, Iron 0.31mg,

Magnesium 17mg, Phosphorus 34mg, Potassium 312mg, Sodium 3mg, Zinc 0.14mg, Manganese 0.098 mg (NDB, 2011). For the successful cultivation of kiwifruit in hilly conditions of Uttarakhand some high quality new varieties are required to evolve through proper breeding programmes. But there is a lack of proper breeding programme and knowledge about pollen morphology, viability and pollen germination in kiwifruit particularly for the conditions of Uttarakhad hills. The present investigation was there undertaken to study about the pollen morphology, viability and germination in kiwifruit grown under hilly conditions of Uttarakhand.

MATERIALS AND METHODS

Pollen Collection

During the peak period of flowering in both the male and hermaphrodite flowers, pollen grains were collected in the petridis.

Pollen Morphology

Size of pollen grains measured in different medium, viz., water, acetocarmine, glycerin jelly and without any medium (dry condition) with the help of ocular micrometer indexed against stage micrometer. The average size (length and breadth) of 50 pollen grains under each medium was considered to arrive at the final value.

Pollen Viability and Longevity

The viability of fresh pollen grains of both the male and hermaphrodite kiwifruit flower was estimated separately by acetocarmine test. For longevity study, the fresh pollen grains of both the male and hermaphrodite flowers were stored in dry specimen tubes, covered with cotton plugs and maintained at room temperature.

$$\textbf{Pollen viability} = \times 100 \quad \frac{\text{Number of stained pollen grains}}{\text{Total number pollen grains observed}}$$

Pollen Germination

The fresh pollen grains of the both the male and hermaphrodite flowers were planted in artificial sucrose media (strength 5%, 10% and 15%), boric acid (strength 0.2%, 0.3% and 0.5%) and in combination of both. Distilled water served as control. "Sitting Drop" culture method (Shivanna and Rangaswamy, 1992) was employed for pollen germination studies. Slides were examined at 6, 12, 18 and 24 hours, after planting the pollen grains in different media and observations on pollen germination and pollen tube length were recorded at least under ten different microscopic fields to calculate the average values.

$$\textbf{Pollen germination} = \times 100 \quad \frac{\text{No. Of germinated pollen grains}}{\text{Total no. of pollen grains observed}}$$

RESULTS AND DISSCISSIONS

Pollen Morphology

Pollen grains of both male and hermaphrodite flower, obtained bar to ovoid shape under dry condition while in liquid medium (water and acetocaramine) and gel medium (glycerin) they obtained round shape. The most of

hermaphrodite pollens shows shrunken inner content because they are empty while the male pollen doesn't show any shrunken inner content in any liquid or gel medium (Table 1).

The maximum length of male and hermaphrodite flower pollen grains (186.28 µ and 113.34 µ respectively) was obtained under T_1 (dry condition) treatment which was statistically at par with T_4 (water) with 159.34 µ in male and 113.34 µ in hermaphrodite flower pollen. While T_4 (water) gave maximum width of pollen grains i.e., 158.71 µ for male flower pollens and 112.73 µ for hermaphrodite flower pollen respectively which was statistically at par with 116.49 µ and 74.73 µ respectively under T_3 (glycerin). Li *et al.* (1989); and Kang *et al.* (1993) also obtained the same results that pollen of Actinidiaceae is remarkably uniform and rather unspecialised. Devi *et al.*, (2015) observed that the shape of the pollen of kiwifruit was pyramidal to ovate when dry and became more spherical when hydrated or with the addition of Acetocarmine solution. Similar observations were also made by Hopping and Jerram (1979).

Pollen Viability and Longevity

It was evident from the Table 2 that, the viability of pollen grains was high. The viability of fresh pollen grain was recorded 100%. The germinability of pollen grains of male flower stored under room conditions have been presented in Table 2 which indicate that after 3 days of storage, the viability of the pollen grains totally declined in male flowers. But, in hermaphrodite flower the viability decreases after 2 days. Hopping (1981) reported that in general, viability of pollen of kiwifruit flower from early season clones exceeds 80 % while that of pollen from mid to late season clones range from 65 75%.

Pollen Germination

Male Flower

Among all the treatments, the maximum pollen germination percentage (91.60%) and pollen length (2483.10 µ) was observed in T_9 (Sucrose 15% + Boric acid 0.5%) after 24 hours and in the treatment combinations of T_1 (sucrose 5%), T_4 (Boric acid 0.2%), T_7 (Sucrose 5% + Boric acid 0.2%) and control (water), no pollen germination was recorded after an interval of 6 hours (Table 13.) and the minimum pollen germination (5.20%) was observed in T_4 (0.2% boric acid) after 12 hours.

Data presented in the Table 3. reveals that after 6 hours of slide preparation highest germination (74.40%) and longest pollen tube (250.98 µ) was obtained under T9 (15% sucrose and 0.5% boric acid) which was followed by 65.60 % under T3 (15% sucrose), 52.40% under T8 (10% sucrose and 0.3% boric acid) for pollen germination and 197.58 µ under T8 (10% sucrose and 0.3% boric acid), 173.55 µ under T6 (0.5% boric acid) respectively. While lowest pollen germination (30.40%) was recorded under T5 (0.3% boric acid) and minimum pollen tube length (138.84 µ) was observed under T2 (10% sucrose) which was statistically at par with T6 (0.5% boric acid) i.e., 41.20%, T2(10% sucrose) i.e., 44.80% for pollen germination and T3 (15% sucrose) i.e., 149.52 µ, T5 i.e., 154.86 µ for pollen tube length.

When the slides were further observed after 12hour interval T_9 (15% sucrose and 0.5% boric acid) treatment with 74.40% pollen germination and 630.12 µ long pollen tube ranked first and found statistically at par with T_3 (15% sucrose), T_2 (10% sucrose) and T_8 (10% sucrose and 0.3% boric acid) for pollen germination (69.20%, 52.40% and 52.40% respectively) and T_8 (10% sucrose and 0.3% boric acid), T_6 (0.5% boric acid) i.e., 558.03 µ and 528.66 µ for pollen tube length respectively. Whereas under T_{10} (water) treatment pollen grains were again fails to germinate which was statistically at par with T_1 (3% sucrose) and T_4 (0.2% boric acid) i.e., 3.60%, 5.20% and 90.78 µ, 106.80 µ for both pollen germination

and pollen tube length respectively.

It is further evident from the data of Table 3 that, after 18 hours of slide preparation highest pollen germination (81.60%) and pollen tube length (1300.29 µ) was observed under T_9 (15% sucrose and 0.5% boric acid). With respect to pollen germination T_3 (15% sucrose) and T_8 (10%sucrose and 0.3% boric acid) with 77.20% and 68.00% germination respectively were found statistically at par with T_9, while for pollen tube length with 1246.89 µ and 1177.47 µ treatments T_8 (10% sucrose and 0.3% boric acid) and T_6 (0.5% boric acid) respectively found statistically at par with the T_9. Still there is no germination was observed under the treatment T_{10} (water) followed by pollen germination of 5.60% and 9.20% under the treatments T_4 (0.2% boric acid) and T_1(5%sucrose) and pollen tube length of 312.39 µ and 36.42 µ under the treatments T_4 (0.2% boric acid) and T_7(5%sucrose and 0.2% boric acid) respectively.

When these treatments are observed after 24 hours, it was recorded that that the treatment T_9 (15% sucrose and 0.5% boric acid) given the best results with highest germination of (91.60%) and maximum pollen tube length (2,483.10 µ) which was followed by T_8 (10% sucrose and 0.3% boric acid) for both pollen germination (90.80%) and pollen tube length (2330.91 µ). It is also observed that pollen grains were still failed to germinate under treatment T_{10}(water) which was followed by 16.00%, 22.00% of pollen germination and 528.66 µ, 587.40 µ of pollen tube length under the treatments T_1(5% sucrose) and T_4(0.2% boric acid) respectively.

Hermaphrodite Flower

The data on pollen germination and pollen tube growth of hermaphrodite flower was presented in Table 14 which provides the evidence that highest pollen germination (25.20%) and pollen tube growth (550.02 µ) was obtained under the treatment T_9 (15% sucrose and 0.5% boric acid) after a time interval of 24 hours. Whereas no pollen germination was observed under any treatment combinations after an interval of 6 hours.

Table 14 is providing the information that the highest pollen germination (4.20%) and longest pollen tube growth (77.43 µ) was observed under the treatments T_7 (5% sucrose and 0.2% boric acid) which was statistically at par with T_2, T_6, T_9 and T_8 (10% sucrose, 0.5% boric acid, combination of 15% sucrose and 0.5% boric acid, 10% sucrose and 0.3% boric acid) i.e., 4.00%, 4.00%, 4.00% and 3.40% respectively for pollen germination and T_4, T_5 (0.2% boric acid, 0.3% boric acid) which was recorded 64.08 µ, 50.73 µ for pollen tube growth respectively. There was no pollen germination observed under T_{10} (water) which was followed by T_1, T_4 and T_5 (5% sucrose, 0.2% boric acid and 0.5% boric acid) i.e., 2.40%, 3.00% and 3.00% for pollen germination and T_2, T_3 (10% sucrose, 15% sucrose) i.e., 24.03 µ, 26.70 µ respectively after a time interval of 12 hours.

When the treatments were observed after an interval of 18 hours (Table 14.), T_5 (0.3% boric acid) showed highest pollen germination (16.00%) and T_9 (15% sucrose and 0.5% boric acid) showed both maximum pollen germination (16.00%) and pollen tube growth (232.29 µ). T_6 (0.5% boric acid), T_4 (0.2% boric acid) showing the 14.80%, 13.60% of germination and T_4 (0.2% boric acid), T_3 (15% sucrose) showing 197.58 µ, 189.57 µ pollen tube growth which were following the highest germination percentage and maximum pollen tube growth respectively. Even after 18 hours of treatment, T_{10} (water) was unable to show any sign of germination. Minimum pollen germination and pollen tube growth (i.e., 6.40%, 7.20% and 168.21 µ, 17088 µ) was observed in the treatment combination of T_1 (5% sucrose), T_2 (10% sucrose) for pollen germination and T_2 (10% sucrose), T_7 (5% sucrose and 0.2% boric acid) for pollen tube growth respectively.

In case of 24hour study (Table 14.) of pre prepared slides for pollen germination and pollen tube growth, highest germination (25.20%) was obtained under the treatment T_9 (0.5% boric acid and 15 % sucrose) which was followed by T_4 (0.2% boric acid) and T_6 (0.5% boric acid) i.e., 24.80% and 22.40% respectively. Highest pollen tube growth (550.02 µ) was obtained under the treatment T_9 (0.5% boric acid and 15 % sucrose) which was followed by T_8 (0.3% boric acid and 10 % sucrose), T_7 (0.2% boric acid and 5 % sucrose) which was showing the length of 509.97 µ, 488.61 µ respectively. When the treatments were subjected to further investigation, it was found that no germination was observed under the treatment T_{10} (water). The treatment T_1 (5% sucrose), T_2 (10% sucrose) were showing the lowest germination of 10.80%, 16.40% and treatments T_1 (5% sucrose), T_5 (0.3% boric acid) were showing the minimum pollen tube growth of 333.75 µ, 373.80 µ respectively.

In the present investigation of male pollen, it was observed that the 15% sucrose solution in combination with 0.5% boric acid was more suitable for pollen germination and pollen tube growth for the pollen grains of male flower. In the comparison study of male and hermaphrodite pollen grains (Table 3. and Table 4.) showed that the vigor of male pollen germination and tube growth is maximum compared to the hermaphrodite pollen germination and tube growth.

The findings of present investigation with respect to pollen germination are supported by the findings of Holocroft and Allan, 1991; Gonzalez et al. 1994; Korkutal et al. 2004 who reported highest pollen germination in 10 % sucrose followed by 15 % and thereafter there was a deleterious effect on the germination probably due to plasmolysis which was also supported by the findings of Borghezen et al. (2011) which illustrated the maximum pollen germination (52.76 %) of Tomuri cultivar in 10 % sucrose solution.

CONCLUSIONS

From present investigation is concluded that the pollen of kiwifruit are highly viable and remain viable for 3-4 days and for artificial pollen germination 15% sucrose solution with 5% boric acid prove to be the best medium.

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APPENDICES

Table 1: Morphology of Pollen Grains at Different Conditions

Treatments	Male Flower Pollen Grain		Hermaphrodite Flower Pollen Grain	
	Length (µ)	Width (µ)	Length (µ)	Width (µ)
T1 - Dry condition	**186.28**	75.29	**119.47**	57.83
T2 – Acetocaramie	129.17	128.07	99.85	98.35
T3 - Glycerine	142.04	**140.49**	104.79	**104.08**
T4 - Control (Water)	**159.34**	**158.71**	**113.34**	**112.73**
C.D. (0.05)	16.653	6.685	7.628	7.305

Table 2: Pollen Viability and Longevity

Days of Storage	Male Flower Pollen Grain (%)	Hermaphrodite Flower Pollen Grain (%)
Ist Day	100	100
IInd Day	37.44	0.32
IIIrd Day	18.92	0.22
IVth Day	6.12	0
Vth Day	0	0

Table 3: Pollen Germination of Male Flower at Different Time Interval

Treatment	Pollen Germination Percentage (%)				pollen Tube Length (µ)			
	6 Hrs	12 Hrs	18 Hrs	24 Hrs	6 Hrs	12Hrs	18Hrs	24 Hrs
T1 - Sucrose (5%)	0.00	3.60	9.20	16.00	0.00	90.78	360.45	528.66
T2 - Sucrose (10%)	44.80	**52.40**	61.20	**85.60**	138.84	339.09	1,025.28	1,735.50
T3 - Sucrose (15%)	**65.60**	**69.20**	**77.20**	80.80	149.52	475.26	1,086.69	1,858.32
T4 - Boric acid (0.2%)	0.00	5.20	5.60	22.00	0.00	106.80	312.39	587.40
T5 - Boric acid (0.3%)	30.40	30.40	48.40	81.600 ± 1.72	154.86	491.28	1,134.75	**2,090.61**
T6 - Boric acid (0.5%)	41.20	41.20	63.60	85.20	**173.55**	**528.66**	**1,177.47**	2,085.27
T7 - Sucrose (5%) + Boric acid (0.2%)	0.00	5.60	10.80	31.20	0.00	178.89	336.42	590.070
T8 - Sucrose (10%) + Boric acid (0.3%)	**52.40**	**52.40**	**68.00**	**90.80**	**197.58**	**558.03**	**1,246.89**	**2,330.91**
T9 - Sucrose (15%) + Boric acid (0.5%)	**74.40**	**74.40**	**81.60**	**91.60**	**250.98**	**630.12**	**1,300.29**	**2,483.10**
T10 - Control	0.000	0.000	0.000	0.00	0.00	0.00	0.00	0.00
$CD_{0.05}$	4.474	4.975	4.177	4.821	14.019	70.306	24.701	64.995

Table 4: Pollen Germination of Hermaphrodite Flower at Different Time Interval

Treatment	Pollen Germination Percentage (%)				Pollen Tube Length at Different Time Interval (µ)			
	6 Hrs	12 Hrs	18 Hrs	24 Hrs	6 Hrs	12 Hrs	18 Hrs	24 Hrs
T1 - Sucrose (5%)	0	2.40	6.400	10.80	0	37.38	178.89	333.75
T2 - Sucrose (10%)	0	**4.00**	7.200	16.40	0	24.03	168.21	397.83
T3 - Sucrose (15%)	0	3.20	9.600	21.60	0	26.70	**189.57**	432.54

Table 4: Contd.,								
T4 - Boric acid (0.2%)	0	3.00	**13.60**	**24.80**	0	**64.08**	**197.58**	427.20
T5 - Boric acid (0.3%)	0	3.00	**16.00**	17.60	0	**50.73**	184.23	373.80
T6 - Boric acid (0.5%)	0	**4.00**	**14.80**	**22.40**	0	**77.43**	178.89	451.23
T7 - Sucrose (5%) + Boric acid (0.2%)	0	**4.20**	12.40	17.60	0	29.37	170.88	**488.61**
T8 - Sucrose (10%) + Boric acid (0.3%)	0	**3.40**	13.20	22.00	0	34.71	184.23	**509.97**
T9 - Sucrose (15%) + Boric acid (0.5%)	0	**4.00**	**16.00**	**25.20**	0	37.38	**232.29**	**550.02**
T10 - Control	0	0.00	0.00	0.00	0	0.00	0.00	0.00
$CD_{0.05}$	0	N/A	3.736	5.157	0	42.716	30.972	59.799